SYNOPSIS

Chemotherapeutic agents have been widely used in the treatment of cancer An ideal chemotherapeutic agent should be selectively toxic to cancer cells Most of the known anticancer agents interfere with the metabolism of host resulting in toxic effects Azidothymidine (AZT) inhibits human immunodeficiency virus (HIV) replication and has been used for the treatment of patients with acquired immunodeficiency syndrome (AIDS) 5 Fluorouracil (5 FU) has been extensively used in the treatment of human carcinomas of the breast head neck and the gastrointestinal tract However AZT and 5 FU are toxic to normal cells It has been reported that the combination of AZT and 5 FU exerts tissue-specific cytotoxic effects correlating with tissue differences in pyrimidine salvage Biochemical studies also revealed that 5-FU enhances the incorporation of AZT into cellular nucleic acids in a tissue specific manner The site specific delivery of drugs decreases cytotoxicity to normal cells Targeted chemotherapy involves the specific carrier mediated delivery of chemotherapeutic agents to tumors and other target tissues Among the various drug delivery carriers liposomes have been extensively used as vehicles for drug delivery However liposomes as drug carrier systems are not target specific In order to confer target specificity immunoliposomes have been prepared by covalently coupling specific antibody to the surface of liposomes and used for drug delivery The present study was undertaken to investigate (1) the effect of AZT and 5 FU either alone or in combination on Avian Myeloblastosis Virus (AMV) infected chicks (2) the effect of AZT and 5 FU either alone or in combination on the multiplication of AMV (3) the effect of 5 FU and AZT encapsulated liposomes and immunoliposomes on AMV infected chicks (4) the apoptotic mode of cell death induced by AZT and 5 FU in Sp2/0 cells grown *in vitro* and AMV transformed myeloblasts *in vivo*

Effect of Azidothymidine and 5 Fluorouracil on Avian Myeloblastosis Virus infected chicks

When AMV infected chicks were treated with AZT 300 mg/kg body weight in 6 equal doses at 1 h day 1 day 2 day 3 day 4 and day 5 p i 2/6 chicks survived on 30th day p i All the infected control chicks died on 17th day p i Increase in the life span of AMV infected chicks was observed when AZT was administered at intervals from 1 h to day 5 p i The LD_{50} value of 5 FU in control chicks was found to be 250 mg/kg body weight When AMV infected chicks were treated with 5 FU 100 mg/kg body weight in 2 equal doses on 7th and 8th day p i 3/6 chicks survived on 30th day p i When AMV infected chicks were treated with AZT 300 mg/kg body weight in 6 equal doses at 1h day 1 day 2 day 3 day 4 and day 5 p i and with 5 FU 100 mg/kg body weight in 2 equal doses on 4th and 5th day p i 3/6 chicks survived on 30th day p i as compared to chicks treated with either AZT (2/6 chicks) or 5 FU (1/6 chicks)

Effect of Azidothymidine and 5 Fluorouracil on the multiplication of Avian Myeloblastosis Virus

The isolation of AMV envelope glycoprotein gp80 purification and characterization of polyclonal and monoclonal anti AMV gp80 antibodies are described The monoclonal anti AMV gp80 D_7B_{10} antibody recognized the AMV and the surface protein gp80 in AMV transformed cells A quantitative antigen capture ELISA for estimating AMV gp80 at ng/ml level in plasma samples and cell culture supernatants was developed using polyclonal and monoclonal anti AMV gp80 antibodies This assay is more sensitive and specific than ATPase assay and used for monitoring infection and disease development in AMV infected chicks AMV concentration and reverse transcriptase activity decreased in infected chicks treated with AZT and 5-FU either alone or in combination A decrease in AMV concentration was observed in culture supernatants of AMV transformed myeloblasts treated with AZT and 5 FU either alone

or in combination In all the cases AMV concentration and reverse transcriptase activity were found to be significantly less in AMV infected chicks treated with AZT and 5 FU in combination as compared to chicks treated with either AZT or 5 FU

Immunotargeting of Azidothymidine and 5-Fluorouracil against Avian Myeloblastosis Virus infection in chicks

Liposomes and immunoliposomes were used as delivery systems for targeting AZT and 5 FU against AMV infection in chicks Liposomes were prepared by mixing egg phosphatidylcholine cholesterol and stearylamine in 51 21 15 ratio The specificity of interaction of immunoliposomes with AMV was ascertained by sucrose gradient analysis The binding of immunoliposomes to AMV transformed myeloblasts was inhibited when the myeloblasts were preincubated with homologous antibody AZT and 5 FU were separately encapsulated in liposomes and immunoliposomes When AMV infected chicks were treated with AZT encapsulated immunoliposomes 300 mg/Kg body weight in 6 equal doses at 1 h day 1 day 2 day 3 day 4 and day 5 p i 4/6 chicks survived on day 30 p i When AMV infected chicks were treated with 5-FU encapsulated immunoliposomes 100 mg/kg body weight in 2 equal doses on 7 and 8 day p i 5/6 chicks survived on day 30 p i A decrease in AMV concentration and reverse transcriptase activity was observed in AMV infected chicks treated with AZT or 5 FU encapsulated liposomes or immunoliposomes In AMV infected chicks treated with immunoliposomes AMV concentration and reverse transcriptase activity were shown to be significantly less as compared to chicks treated with AZT or 5 FU encapsulated liposomes alone or free drug

Apoptosis induced by Azidothymidine and 5-Fluorouracil in Sp2/0 cells grown *in vitro* and in AMV transformed myeloblasts *in vivo*

Azidothymidine and 5-Fluorouracil induced characteristic apoptotic mode of cell death in mouse myeloma cell line Sp2/0 *in vitro* and in AMV transformed myeloblasts

in vivo Viability of Sp2/0 cells decreased progressively upon treatment of cells with increasing concentrations of AZT or 5 FU Phase contrast and propidium iodide stained fluorescence micrographs of AZT and 5 FU treated Sp2/0 cells revealed typical apoptotic characteristics such as decrease in cell size presence of blebbs in plasmamembrane formation of apoptotic bodies and chromatin condensation Frequency distribution histograms indicated significant decrease in S phase cells with concomitant appearance of cells having fractional DNA content typical of apoptosis DNA isolated from AZT and 5 FU treated Sp2/0 cells showed oligonucleosomal ladder bands (180-200 bp) characteristic of apoptosis DNA fragmentation induced by AZT was protected when Sp2/0 cells were treated with AZT in presence of thymidine or uridine 5 FU induced DNA fragmentation was protected when cells were treated with 5 FU in presence of thymidine but not in presence of uridine Increase in endonuclease activity was observed in the nuclear extract of Sp2/0 cells treated with either AZT or 5 FU Poly(ADP ribose) polymerase activity increased when Sp2/0 cells were treated with increasing concentrations of AZT or 5 FU

Nuclear fragmentation was observed in myeloblasts isolated from AMV infected chicks treated with either AZT or 5 FU DNA isolated from these myeloblasts also showed oligonucleosomal ladder bands (180-200 bp) Increase in endonuclease activity was observed in nuclear extract of myeloblasts isolated from AMV infected chicks treated separately with AZT and 5 FU

The results of the present study are summarized as follows

1 AZT was shown to be more effective when AMV infected chicks were administered with AZT at intervals from 1 h to day 5 p i 5 Fluorouracil was shown to be more effective when chicks were treated at the onset of leukemic symptoms i e 7th and 8th day p i AZT and 5 FU in combination was shown to be more effective as compared to AZT or 5 FU alone

2 Polyclonal and monoclonal anti AMV gp80 antibodies were purified and characterized Sandwich ELISA was standardized to estimate AMV proteins at ng/ml level This assay is more sensitive and specific than ATPase assay and used to estimate AMV concentration in plasma samples and culture supernatants

3 AMV concentration and reverse transcriptase activity were found to be significantly less in AMV infected chicks treated with AZT and 5 FU in combination as compared to chicks treated with either AZT or 5 FU

4 In AMV infected chicks treated with AZT or 5 FU encapsulated immunoliposomes AMV concentration and reverse transcriptase activity were shown to be significantly less as compared to chicks treated with either AZT or 5 FU encapsulated liposomes or free drug

5 AZT and 5 FU induced apoptosis in Sp2/0 cells grown *in vitro* and in AMV transformed myeloblasts *in vivo* Characteristic apoptotic morphological and biochemical changes were observed

In conclusion AZT or 5-FU encapsulated immunoliposomes were shown to be more effective against AMV infection in chicks as compared to chicks treated with either the drug encapsulated in liposomes or free drug AZT and 5 FU in combination were shown to be more effective as compared to either AZT or 5 FU alone against AMV infection in chicks AZT and 5 FU induced apoptosis in Sp2/0 cells grown *in vitro* and AMV transformed myeloblasts *in vivo*